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Dr. T. M. Sonneborn
Department of Zoology
Indiana University
Bloomington, Ind.

Dear Tracy:

When you were here last we were in the midst of some experiments of whose results we were not at that time certain, and so I hesitated to discuss them until I was more sure of the facts. We have now repeated these experiments sufficiently often to be sure of their reproducibility, and I know that you will be intensely interested in the features of the system which we uncovered. I might note in passing that a long time ago you wrote asking whether the yeast material could be used in a classroom experiment. I believe that the present series of experiments are reduced to an operational level which could easily be handled by students.

The experiments started out fundamentally with the following point of view. It seemed likely that a method of testing the plasmagene hypothesis would be obtained if the following situation were to be true; that there was a gene which duplicated its plasmagene at an extremely low rate as compared with the self-duplicating capacities of the plasmagene. Under such conditions the inheritance of the corresponding character would for the most part be determined by the cytoplasmic components. Now, in view of the haphazard transmission of such cytoplasmic elements, it would be expected that such a character would be much more highly unstable than one in which the transmission was under the direct influence of the gene. On this basis, it was decided to treat haploid cells with mutagenic agents and to isolate mutants, concentrating on the selection of those which were highly unstable. For ease of detection and study of stability, color mutants were looked for.

A set of such characters were found and it is almost certain, from the point of view of the phenotypic characteristics, that it is the same variant isolated by Tatum and used by Lindegren in his recently published paper. As you will note, his interpretation of his segregation results are not cytoplasmic. We found that we could, with the aid of heat-treatments at temperatures and durations which did not lead to a decrease in the viable count, convert 1 out of 10 individuals from the pink

phenotype to the white. Furthermore, we found that we could suppress the appearance of the whites by providing an excess of methionine. Here again the experiments were controlled so that selection is not involved.

Essentially, what was done was to get a known number of cells and plate them on excess methionine plates and normal plates. The same number of clones appear in each type of plate, but the number of whites in the presence of methionine is less than one-tenth of 1% as compared with 25% in the plates with low methionine content. These whites we obtained by plating in the absence of methionine or by heat-treatment of pinks which were then plated on methionine, and required about 200 cell generations on methionine plates to revert to the pink phenotype. We have here then what corresponds to a Dauer modification induced either by heat-treatment or by the absence of a particular substrate in the environment. Except for the ultimate reversion in the presence of substrate, we have then a situation which is analogous to the melibiose situation.

Once this had been established, it was decided to see if we could obtain a purely cytoplasmic type of phenomena by obtaining a situation in which one of the normal plasmagenes mutated in the cytoplasm. This was done by irradiating pinks grown in the presence of methionine under which conditions it would be presumed to have an excess of the pink plasmagene. A red variant was found which was highly unstable and reverted to pink. This red variant was also stabilized by the presence of methionine and in its absence would revert to the extent of 99% of the individuals plated. These red variants, when subjected to the heat-treatment under the same conditions as used for pink to white transformations, reverted to the extent of 70% of the individuals to the pink variety. These pinks derived from the reds have not shown any tendency to revert to the pink. It would appear therefore that we have in the red variant a purely cytoplasmic mutation which can be irreversibly lost by either the absence of substrate or by heat-treatment.

We have also found that the response of this component to temperature is entirely different from that of the cells; that is to say, conditions can be arranged in which the mutation rate from red to pink is a function of the rate of cell division, quite analogous to ~~your~~ experiment with the Kappa. Insofar as the role of substrate in stabilizing the character is concerned, the experiments with the red variants represent a complete analogy with the melibiose case. ~~The experiments are now in a state where I could gain much from an opportunity of discussing~~

the data with you. I am hoping that we will have the opportunity of getting together in Chicago. If you are going, please let me know what days you will be there so that we can arrange a meeting.

Sincerely yours,

S. Spiegelman

sa/b